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Original Article

Synergistic Effects of *Beauveria Bassiana*, Diatomaceous Earth, and Insecticides on Mortality and Enzyme Activities of *Spodoptera Frugiperda* (J.E. Smith)

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Abstract

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of the most destructive pests for many agricultural crops. Since quickly develops resistance in FAW to most classes of pesticides, more effective and safer biological ways to control the pest are needed. Laboratory studies investigated the interaction between the fungal entomopathogen *Beauveria bassiana*, diatomaceous earth (DE) with chlorantraniliprole or emamectin benzoate when applied to 2nd instar FAW larvae. The biological parameters of *B. bassiana* (germination rate and average daily mycelia growth) were not inhibited by chlorantraniliprole and DE treatments, on the contrary the effect of emamectin benzoate. Interaction of *B. bassiana* (1×10^8 conidia/mL), chlorantraniliprole and DE exhibited maximum larval mortality (55–100%), while *B. bassiana* (1×10^4 conidia/mL) alone showed minimum larval mortality (5–29%) recorded at intervals 2–10 days. The results indicated the activities of mixed function oxidase (MFO), glutathione S-transferase (GST) and total esterase (EST) in FAW larvae were significantly lower than that of the control groups at different intervals post-treatment, indicating the inhibitory effect of the all-treated applications. *B. bassiana* combined with chlorantraniliprole and DE had better inhibition effects than applications alone.

Keywords: *Beauveria bassania, Chlorantraniliprole, Detoxification enzymes, Emamectin benzoate, Fall armyworm, Synergism.*

Introduction

FAW, classified as one of the most destructive insect pests plaguing crops worldwide [1]. Poses a formidable challenge to global food security. Its voracious larvae inflict widespread damage across a diverse range of crops, primarily targeting maize where studies estimate yield losses as high as 30-50% [2]. While insecticides have been a cornerstone of crop protection, their effectiveness is declining, and the problem is compounded by numerous environmental and human health concerns associated with their use [3]. As a result, effective management of FAW population has become a complex task.

Therefore, it has become important to find control methods that can protect crops and control the FAW population effectively.

Entomopathogenic fungi show advantages as effective biological control agents, compared to chemical control. They have demonstrably proven their efficacy in curtailing populations of diverse insect pests, including *Spodoptera* species [4, 5]. DE composed of fossilized unicellular diatoms and algae, possesses a remarkable capacity to absorb esters, rendering it a natural insecticide [6, 7]. Numerous studies have reported that the combination of entomopathogenic fungi and DE with insecticides have significantly improved their pest control efficacies [6, 7], the synergistic effects between these tools against FAW have not been reported.

Insects possess remarkable resilience against xenobiotic threats, both natural and synthetic, mediated by various enzyme systems [8]. Among these, EST, GST, and MFO play key roles in insect defense mechanisms [8]. Building upon this knowledge, the present study aimed to evaluate the compatibility of two commonly used insecticides with *B. bassiana*, and the best compatible insecticide was selected to combine treatment with *B. bassiana* and DE to evaluate the synergistic interaction against FAW as well as the effect on detoxification enzymes.

Materials and Methods

Insect Cultures

Larvae of FAW were collected from maize fields in El Senbellawein City, El-Dakahlia Governorate, Egypt (30°52'47.40"N, 31°28'10.12"E) and transported to the laboratory. Larvae were reared continuously on fresh, pesticide-free maize leaves (*Zea mays* L.) in plastic containers (6 L capacity) and covered with a fine mesh to allow for ventilation. These containers were kept at 25 ± 1 °C, 65 ± 5% RH, and with a L 14 h:D 10 h photoperiod in growth chamber (Insect Population Toxicology Department, Central Agricultural Pesticides Laboratory, Egypt). Newly molted second instar larvae of the first generation were used in experiments.

Fungal Preparations

Sampling Sites

The soil was sampled from crop fields from different locations of El Senbellawein City, El-Dakahlia Governorate, Egypt (30°52'47.40"N, 31°28'10.12"E). Three soil samples from each field were taken from 10 cm deep under the earth, and then these samples were mixed together into a single sample. After the collection of the samples, these were preserved at 4 °C until used for isolation of insect pathogenic fungi.

Screening of Soil Samples for Pathogenic Fungi

The soil samples collected from diverse sites were used for the isolate *Beauveria bassiana* (AUMC Accession No: 9896). The medium comprised glucose (10 g), peptone (10 g), and agar (30 g) in 1 L of water. After that, 0.005% tetracycline, 0.06 % streptomycin, 0.005 % cycloheximide, and 0.01% v/v dodine were added. A 10 g soil sample was put into tetrasodium pyrophosphate (100 mL) and was mixed by a magnetic stirrer. The product 100 µL from every sample was spread on the media, and the plates were kept at 27 °C for 10-15 days in dark conditions. The developing colonies were aseptically sub cultures on Czapek-dox agar medium (CZA) in plates; the fungi were examined under a microscope for qualitative conidiogenesis. Detected in Bio-insecticide Production Unit, National Research Centre, Egypt.

The isolates were cultured on (CZA) medium with yeast extract (1%) in Petri dishes (9 cm diam. × 9 cm height) and were grown for 15 to 20 days at 27 °C. The spores were harvested by scraping the surface of 15-20 days old culture gently with a sterilized glass slide. The spores were suspended in distilled water with 0.1% Tween-80® and mixed by a magnetic stirrer for 10 min. After that, five different conidial concentrations of *B. bassiana* (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/mL) were prepared.

Diatomaceous Earth (DE) and Insecticides

The diatomaceous earth **(**DE) formulation used in this experiment was PyriSec® (Natural Source LLC, FL, USA), which contained 97.5% amorphous silicon dioxide. Two insecticide formulations tested recommended by Egyptian Ministry of Agriculture for FAW control; chlorantraniliprole (18.5% SC, Coragen®) and emamectin benzoate (5% SG, Proclaim®).

Conidial Germination and Mycelia Growth Test in The Presence of Insecticides and Diatomaceous Earth (DE)

The influence of chlorantraniliprole, emamectin benzoate and DE on the radial growth of *B. bassiana* was investigated. This was achieved by layering three concentrations of each insecticide (10, 50, and 100 ppm) and DE (200, 400, and 600 ppm) on solidified PDA disks. Each disk comprised 10 mL of PDA medium and was plated in 9 cm Petri dishes that were solidified for 2 h and left to dry overnight. Uncontaminated PDA plates served as the control. Mycelial disks of *B. bassiana* (2 cm diameter) were inoculated onto each treatment and control plate. The entire experimental setup was then incubated under controlled conditions. Colony diameters were meticulously measured on a daily basis for 10 days.

To evaluate the effect on germination, it was combine each concentration of insecticide and DE in 100 ml of sterile Sabouraud Dextrose Agar (SDA) liquid culture medium contained in a 250 ml vial. For each insecticide and DE, uninoculated culture medium served as the control. After the introduction of *B. bassiana* for each treatment, the entire experimental setup was incubated under controlled conditions at 25 ± 1 °C. After an incubation period of 10 days, the number of germinated conidia was accurately counted. Fungal spores that exhibit germ tube lengths exceeding the diameter of their parent spores are classified as spores. The experiment was repeated on three times.

Toxicity of Beauveria Bassiana, Diatomaceous Earth (DE) and Chlorantraniliprole Alone or In Combination

B. bassiana was evaluated at two distinct conidial concentrations: 1×10^4 and 1×10^8 conidia/mL. The lowest concentrations of chlorantraniliprole (10 ppm) and DE (200 ppm) were also employed. Individual larvae were subjected to immersion treatments involving *B. bassiana*, DE, and chlorantraniliprole administered both individually and in various combinations. Following a 24 h incubation period, larvae infected with *B. bassiana* were subsequently exposed to brief immersion treatments (30 s) in chlorantraniliprole and DE solutions. Groups of twenty larvae were transferred to sterile Petri dishes equipped with mesh gauze and rubber bands to impede escape. Untreated leaves were provided daily to sustain the larvae. The experiment was replicated on three times. Mortality rates of the larvae were recorded at 24-h intervals for a period of 10 days.

Distinctive characteristics, such as darkening of the larval bodies and the subsequent emergence of mycelia, facilitated the identification of *B. bassiana*-infected individuals.

Enzyme preparation

Newly molted second instar larvae of FAW were separated and fed on a fresh leaves treated with *B. bassiana*, DE and chlorantraniliprole alone or in combination with each other. For control larvae were fed on a fresh untreated leaves. The treated larvae were placed in plastic cups followed by incubation at 25 ± 1 °C and 65 ± 5% RH. Each treatment consisted of three replicates and each replicate consisted of 10 larvae. Following 2, 4, 6, 8, and 10 days, the larvae were homogenized on ice in homogenization buffer (0.1 M phosphate buffer, pH 7.6, containing 1 mM EDTA, 1 mM DTT, 1 mM PTU, 1 mM PMSF and 20% glycerol). Insects were chilled on ice before homogenization. Homogenized in 2 mL buffer, the homogenate was centrifuged at 4 °C, 10,000 rpm for 15 min. The final supernatants were used as the enzyme preparation.

Enzyme Activity Assays

The assay of mixed function oxidase (MFO) was conducted using the procedures developed by Hansen and Hodgson (1971) [9]. Total esterase (EST) activity was determined using a-naphthyl acetate (a-NA) as a substrate according to the methods of Van Asperen (1962) [10]. Glutathione S-transferase (GST) activity was measured using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate according to the methods of Habig and Jakoby (1981) [11].

Total protein content was determined by the Bradford (1976) [12] method using bovine albumin as a standard.

Data Analysis

The data underwent statistical analysis through the application of a one-way Analysis of Variance (ANOVA) test. Subsequently, pairwise comparisons of the mean values were conducted using Tukey's Honestly Significant Difference (HSD) test, adhering to a significance level of $p < 0.05$. The entirety of the data analysis was executed utilizing the established capabilities of the SPSS 19.0 software.

Results

Radial Growth and Conidial Germination

A statistically significant interaction was observed between the type and concentration of insecticides used, which affected the radial growth of *B. bassiana* during the incubation periods (Figure 1). The highest average daily radial growth was observed in the control while the lowest growth was observed for emamectin benzoate at concentration 100 ppm with an average value of 0.44, 0.32, 0.22, 0.15 and 0.11 mm after 2, 4, 6, 8, and 10 days of incubation, respectively (Figure 1A). None of the applied concentrations of chlorantraniliprole demonstrated a statistically significant effect on the growth when compared to the control across all inoculation days (Figure 1B). Notably, no statistically significant impact on the growth of *B. bassiana* was observed across the employed concentrations of DE, as illustrated in Figure 1C.

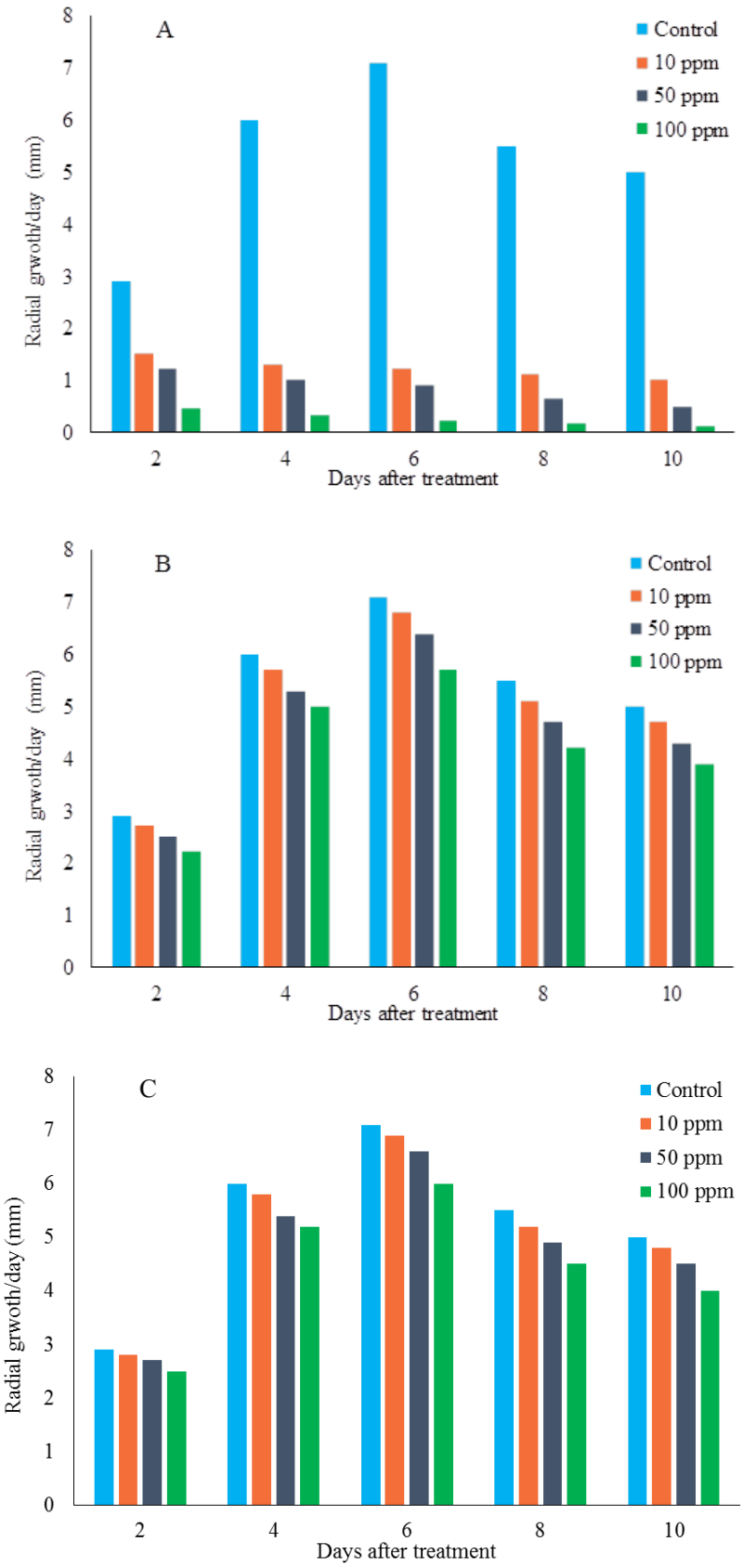

Figure 1 Effect of emamectin benzoate [A], chlorantraniliprole [B] and diatomaceous earth [DE, C] on radial growth of *Beauveria bassiana*

A statistically significant decline in the conidial germination of *B. bassiana* was observed with increasing concentrations of both insecticides employed compared to the control (96.7%), as depicted in Figure 2. Notably, the highest recorded germination rate (91.5, 86.4, and 83.3%) was associated with chlorantraniliprole at concentrations of 10, 50, and 100 ppm, respectively. Conversely, the lowest germination rate (20.1, 14.8%, and 8.3%, respectively) was observed for emamectin benzoate. Regarding the effect of DE on *B. bassiana* a conidial germination was statistically non-significant across the tested concentrations (Figure 2).

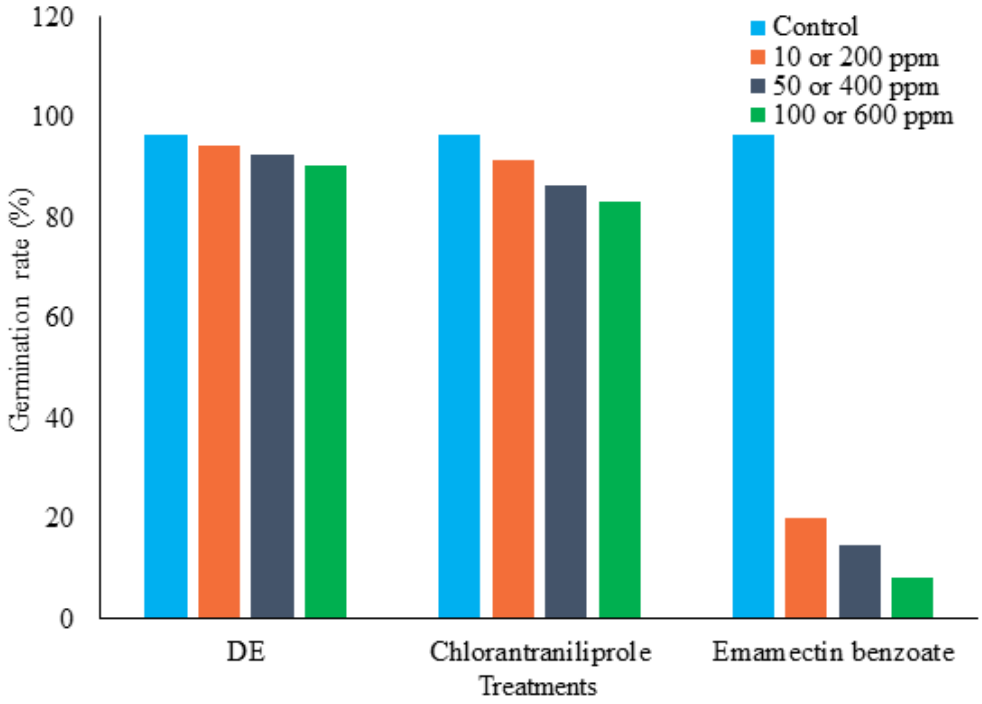

Figure 2 Effect diatomaceous earth (DE, 200, 400, 600 ppm), chlorantraniliprole and emamectin benzoate (10, 50, 100 ppm) on conidial germination of *Beauveria bassiana*.

Toxicity of Beauveria Bassiana, Diatomaceous Earth (DE) and Chlorantraniliprole Alone or In Combination

Mortality in response to different treatments (individual or joint treatments) differed significantly at different time intervals (Table 1). It was observed that the mortality under the combined treatment with *B. bassiana*, DE, and chlorantraniliprole was significantly higher than individual treatments. Moreover, joint all treatments had a significant synergistic interaction at different time interval. The highest mortality rate (100%) was achieved with the combined treatment of *B. bassiana* (1 x 10^8 conidia/mL), DE, and chlorantraniliprole after 8 and 10 days. In contrast, the lowest mortality rates (3.33 and 5.06%) were observed with individual treatments of low-concentration DE and *B. bassiana*, respectively, at the two-days. The results revealed that insects' mortality was dose and time dependent.

Table 1 Mean larval mortality (±SE) of 2nd instar larvae of FAW exposed to *Beauveria bassiana* alone and in combination with diatomaceous earth (DE) and chlorantraniliprole at different intervals

Treatment	Larval mortality (%)					Expected mortality (%)	Co-toxicity factor	Type of action
	2d	4d	6d	8d	10d			
[a]F1	5.06 (1.34)fg	8.75 (1.61)g	13.00 (3.47)gh	21.33 (3.12)g	29.06 (3.33)gh			
[a]F2	8.15 (6.77)f	10.89 (1.82)f	18.44 (1.97)g	26.24 (2.62)f	32.33 (4.21)fg			
[b]DE	3.33 (1.07)fg	5.00 (1.16)gh	10.66 (1.33)h	16.83 (2.50)h	24.17 (1.59)hi			
[c]Chlor	27.17 (1.95)e	37.30 (1.71)e	49.52 (1.01)de	53.65 (2.30)d	57.37 (0.87)d			
DE + Chlor	29.12 (1.84)e	44.16 (1.27)d	54.00 (2.33)d	55.07 (1.73)d	58.54 (2.07)d			
F1+DE	9.87 (0.97)f	12.13 (1.43)f	25.42 (1.96)f	33.16 (3.45)e	36.76 (2.55)f	19.67	-19.44	Add
F2+DE	10.22 (1.50)f	14.42 (0.67)f	27.01 (1.32)f	35.35 (2.04)e	40.86 (1.33)e	23.55	-19.07	Add
F1+Chlor	35.14 (1.48)d	51.79 (1.77)c	60.27 (1.38)c	67.69 (1.61)cd	71.06 (1.46)c	76.80	-18.52	Add
F2+Chlor	40.15 (1.19)bc	54.00 (2.64)c	63.16 (2.62)c	71.13 (1.57)c	74.95 (1.20)c	83.79	-17.34	Add
F1+DE+Chlor	48.22 (1.48)b	66.37 (2.13)b	80.39 (1.39)b	86.21 (1.91)b	91.79 (1.89)b	111.03	-13.30	Add
F2+DE+Chlor	54.69 (2.15)a	79.71 (1.36)a	96.52 (2.18)a	100.0 (0.05)a	100.0 (0.05)a	134.25	-9.39	Add

[a]*Beauveria bassiana* (F1=1 x 10^4, F2=1 x 10^8 conidia/mL)
[b]diatomaceous earth
[c]chlorantraniliprole; Values in a column followed by different lowercase letters are statistically different at the 5% level (Tukey test)

Effects of Beauveria Bassiana, Diatomaceous Earth (DE) and Chlorantraniliprole Alone or In Combination on the Enzyme's Activity

Following larval exposure to treated diets, the activities of GST, EST, and MFO exhibited statistically significant variations between individual and combined treatments across different time points, as depicted in Figure 3. Although enzymes activity generally declined over time compared to the control group, a peak in activity was observed for all treatments after 2-days. Notably, the combined treatment employing a high concentration of *B. bassiana* (1 x 10^8 conidia/mL) alongside DE and chlorantraniliprole yielded the lowest levels of GST, EST, and MFO activities of 20.97, 0.11 and 0.115 U/mg protein, respectively, after 10 days of treatment compared to an activity of 55.78, 2.38 and 0.784 U/mg protein, respectively in the control (Figure 3: A, B, and C).

Discussion

Previous studies by Lacey *et al.* and Ismail [4,13] demonstrated that entomopathogenic fungi cause physiological changes in insects that make them more susceptible to insecticides. However, very few studies have elaborated the possible compatibility of entomopathogenic fungi with diatomaceous earth (DE) as a natural insecticide and insecticides. Therefore, this study was conducted to determine the influence of individual and combined applications of these tools against FAW.

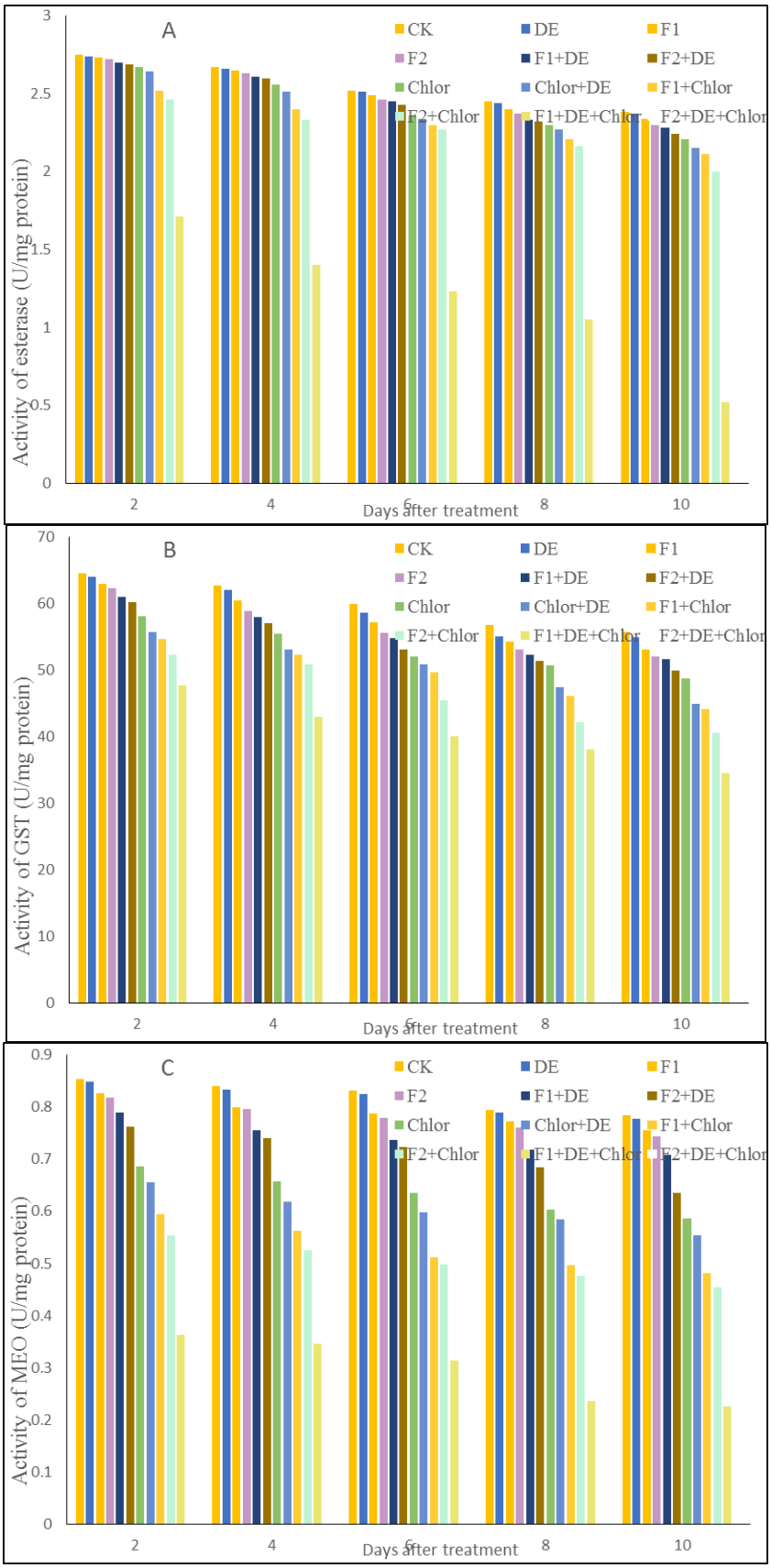

Figure 3 Changes in esterase [A], glutathione S-transferase (GST) [B] and mixed function oxidase (MFO) [C] activity of FAW following treatment with *Beauveria bassiana* (F1, 1 x 10^4 and F2, 1 x 10^8 conidia/mL), diatomaceous earth (DE) and chlorantraniliprole (Chlor) at different time intervals

There was no negative effect of DE and chlorantraniliprole on the growth and germination conidia of *B. bassiana*, and the strong compatibility indicates the potential for combined use for effective FAW control. While the high inhibition of *B. bassiana* by emamectin benzoate indicates incompatibility. The current study's findings regarding the differential compatibility of emamectin benzoate, chlorantraniliprole, and DE with *B. bassiana* align demonstrably with previous research conducted by Ismail *et al.* [14]; Zhang *et al.* [15] who observed similar effects of emamectin benzoate on growth and conidial germination of *B. bassiana*. Ismail *et al.* [14]; Jia *et al.* [16] who observed that different concentrations of chlorantraniliprole did not affect the growth and germination of *Metarhizium anisopliae* and *B. bassiana*. Likewise, the mycelial and conidial germination of *B. bassiana* were higher at lower doses of DE than at higher doses [6, 17].

The current study's findings demonstrably support the existence of a synergistic effect between *B. bassiana* and both chlorantraniliprole and DE, leading to significantly increased mortality of FAW. This synergy is likely attributed to the stress-inducing capabilities of the insecticides and DE, which compromise the immune response of the FAW and enhance their susceptibility to the fungal pathogen. These results are consistent with Nozad-Bonab *et al.* [18], who reported higher mortality of *Tuta absoluta* (Meyrick) when *B. bassiana* was combined with chlorantraniliprole. Additionally, Jia et al. [16]; Wakil *et al.* [19] reported a significant reduction in the number of *Helicoverpa armigera* (Hübner) larvae in combined treatments of *M. anisopliae* and chlorantraniliprole compared to their individual applications**.** Moreover, Riasat, *et al.* [6]; Pourian and Alizadeh [17] demonstrated additional reactivity in *B. bassiana* and DE against *Rhyzopertha dominica* (Fab.), *Callosobruchus maculatus* (F.) and *Oryzaephilus surinamensis* (L.).

The observed reduction in enzyme activity suggests a multi-pronged attack on the FAW's defense mechanisms. *B. bassiana* infection likely compromises the insect's immune system, rendering it more susceptible to the toxic effects of both DE and chlorantraniliprole. This synergistic interplay between the three agents effectively overwhelms the FAW's detoxification pathways, leading to its demise. These results are in line with the findings of Wu *et al.* [20] who observed the reduction in enzymes activity of *S. litura* (F.) following *B. brongniartii* and matrine application. Furthermore, Jia *et al.* [16] who also observed a similar reduction in GST and EST activity of *L. migratoria* in response to individual or combined treatments of *M. anisopliae* and chlorantraniliprole.

Conclusion

In summary, the results suggest that combined treatments of DE, chlorantraniliprole and *B. bassiana* cause significant reduction in larvae mortality (%) of FAW. This strong synergistic effect of the mixture treatments is possibly related to the changes in MFO, GST, and EST activities in FAW. Therefore, these results have revealed the biochemical processes involved in the strong synergistic action of chlorantraniliprole, DE and *B. bassiana* to overcome this defense strategy of FAW. Such information is useful in designing integrated pest control programs against FAW through a promising synergistic mechanism between DE, chlorantraniliprole and *B. bassiana*, representing an alternative strategy for the control of this pest.

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